The Report committee for Andrew Peter Lange

Certifies that this is the approved version of the following report:

Assessment of Various Mechanisms Involved in Heat-Stress Induced Reductions in Orthostatic Tolerance

Approved By

Supervising Committee:

Supervisor: _____

Robert M. Brothers

_____

Roger Farrar

Assessment of Various Mechanisms Involved in Heat-Stress Induced Reductions in Orthostatic Tolerance

By:

Andrew Peter Lange, B.S.

Report

Presented to the Faculty of the Graduate School

Of The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science in Kinesiology

The University of Texas at Austin

August 2013

ACKNOWLEDGMENTS

I would like to thank my advisor Dr. Brothers for his help and expertise in completing my graduate program. I would like to thank Josh Lee for his excellent research that I have been privileged to be a part of the last couple years. I would also like to thank Kevin Christmas for his constant help throughout my Masters' program and with this project.

Abstract

Assessment of Various Mechanisms Involved in Heat-Stress Induced Reductions in Orthostatic Tolerance

Andrew Peter Lange, M.S. Kin

The University of Texas at Austin, 2013

SUPERVISOR: Robert M. Brothers

Purpose: This study aimed to expand our knowledge of the underlying mechanisms of orthostatic tolerance. First, cerebral perfusion was compared with reductions in orthostatic tolerance between normal thermic and heated conditions. The researchers' hypothesized that subjects with the greatest reduction in orthostatic tolerance will experience the largest drop in cerebral blood flow. Additionally, ANG II was measured in order to identify if during passive heating, the elevation in plasma ANG II is negatively correlated with heat-stress induced reductions in orthostatic tolerance. Lastly, orthostatic tolerance changes during the simulated hemorrhage between heat stress and normal thermic conditions will be compared to fitness level, measured by VO2 max.

Results and Conclusion: Cerebral perfusion, as indexed by middle cerebral artery blood velocity, was reduced during heat stress compared with normothermia ($P < 0.001$); however, the magnitude of reduction did not differ between groups ($P = 0.51$). In the initial stage of LBNP during heat stress (LBNP 20 mmHg), middle cerebral artery blood velocity and end-tidal PCO_2 were lower; whereas, heart rate was higher in the large difference group compared with small difference group ($P < 0.05$ for all). In opposition to the hypotheses, the large differences in tolerance to a simulated hemorrhage during normothermic and heat stress conditions are not solely related to the degree of heat stress-induced reduction in cerebral perfusion. Also, an individuals' level of cardiorespiratory capacity (fitness) and/or the degree of heat stress-induced increase in plasma ANG II does not reliably predict the level of reduction in tolerance to a simulated hemorrhage challenge when heat stressed.

TABLE OF CONTENTS

List of Figures

List of Tables

Introduction:

Exercise Physiology studies the physiological changes the body undergoes during exercise, an action that poses a significant challenge to the body's maintenance of homeostasis. Due to the dramatic movements the body goes through while exercising, taking precise measurements can be difficult. Another perturbation that poses a significant stress to the body that humans are frequently exposed to is orthostatic stress. One common approach to investigate the response to orthostatic stress is using the application of lower body negative pressure, which safely simulates a hemorrhaging situation. When the human body experiences an increase in internal temperature either due to exercise, or exposure to elevated environmental temperatures, the strain to the body is exacerbated. when exercising from increases in metabolism. Wilson (2006) found that during heat stress, subjects experienced reduced blood pressure control and reduced tolerance to the physiological challenge of a simulated hemorrhage. Subjects who are able to tolerate the simulated hemorrhage for longer period of time display high levels of orthostatic tolerance. The opposite is true for subjects who quickly fail during the simulated hemorrhage, they display orthostatic intolerance.

Orthostatic intolerance is the body's inability to control blood pressure, normally during the standing process. Going from the seated to a standing position presents a drastic change to the human body. It results in a gravity dependent reduction in central blood volume and a subsequent reduction in cardiac output. As a result there is a slight drop in arterial blood pressure and cerebral blood flow. Ultimately a reduction in cerebral blood flow below a critical threshold results in a loss of consciousness. The exact physiological mechanisms that reduce orthostatic tolerance are still unknown and are likely multifactorial. Keller, et al. (2009) examined the differences between orthostatic tolerance in subjects who were heat stressed on one day, and

tested under normal thermic conditions on another. Subjects showed an 80% reduction in orthostatic tolerance during the heat stress test. In order to test whether heat stress induced reductions in central blood volume was one of the contributing factors to reduced orthostatic tolerance, researchers expanded subjects' blood volume, using intravenous infusion of a dextran solution, until central venous pressure was returned back to its normothermic baseline value during another day of heat stress. By expanding subjects' blood volume, the reduction in orthostatic tolerance was completely eliminated. These results point to heat stress induced reductions in central blood volume as being one of the contributing mechanisms to the loss of orthostatic tolerance during heat stress.

A reduction in cerebral blood flow is a classic marker of orthostatic intolerance and syncopal (fainting) symptoms will always occur if cerebral blood flow is not sufficiently maintained. The brain needs constant and consistent blood flow, which provides oxygen and substrate delivery to operate properly. In 2009, Brothers investigated whether heat-stress induced reductions in arterial carbon dioxide partial pressure (hypocapnia) was one of the primary underlying mechanisms that reduced cerebral blood flow during heat stress. Results found that when the reduction in arterial carbon dioxide partial pressure during heat stress was prevented there was an approximate 50% attenuation of the reduction in cerebral blood flow; however, it still was reduced significantly relative to normal thermic conditions. These results point to reductions in arterial carbon dioxide partial pressure as a contributing factor to reductions in cerebral blood flow during heat stress, but it is not the primary mechanism that controls cerebral blood flow.

Although orthostatic intolerance is accentuated in heat stress tests, subjects with extremely poor orthostatic tolerance can be affected during normal temperatures as well.

Greenleaf (2000) performed LBNP tests on subjects during normal thermic conditions. The research was geared towards investigating the role of angiotensin II (ANG II), a potent vasoconstrictor, as an underlying mechanism that affects orthostatic intolerance. Results showed that subjects with attenuated activation of the renin-angiotensin-aldosterone system (RASS) displayed the highest levels of orthostatic intolerance. These results are similar to those found by Harrison (1985) and Jacob (1997), both of whom found that a reduced RASS response was directly linked to orthostatic intolerance. Additionally, Escourrou (1982) found that heat stress activates the RASS. Since increased activation of RASS improves orthostatic tolerance during normal thermic conditions, it stands to reason that during heat stress, participants with the greatest magnitude of RASS activation would be the most orthostatically tolerant.

The renin-angiotensin-aldosterone system is not the only pathway investigated for connections to orthostatic tolerance. Levine (1991) researched the connection between orthostatic tolerance and max oxygen consumption (VO2 max). The results showed that variation in the carotid baroreflex between subjects did a better job of explaining the differences in orthostatic tolerance than VO2 max. There are many mechanisms proposed to affect orthostatic tolerance, many of which are related to fitness level, but there is not a linear relationship between fitness level and orthostatic tolerance. These results have also been shown by Mtinangi (1999) and Winker (2005), both of whom found no linear relationship between fitness level and orthostatic tolerance. However, just because researchers have not identified a direct relationship between the two, this does not exclude it from possibility. Furthermore, none of these studies were performed in hyperthermic individuals and therefore the interaction between elevated internal temperatures, aerobic fitness, and orthostatic tolerance remains unknown.

This study aimed to expand our knowledge of the underlying mechanisms of orthostatic tolerance. First, cerebral perfusion was compared with reductions in orthostatic tolerance between normal thermic and heated conditions. The researchers' hypothesized that subjects with the greatest reduction in orthostatic tolerance will experience the largest drop in cerebral blood flow. Additionally, ANG II was measured in order to identify if during passive heating, the elevation in plasma ANG II is negatively correlated with heat-stress induced reductions in orthostatic tolerance. Lastly, orthostatic tolerance changes during the simulated hemorrhage between heat stress and normal thermic conditions will be compared to fitness level, measured by VO2 max.

Methods:

All experimental protocols were approved by the International Review Board at the University of Texas at Austin. All subjects were informed of procedures and risks, verbally and in written consent, before they began the study.

Twenty participants (twelve men) were recruited for this study. Subjects were non-smokers and had normal blood pressure. Nine subjects were highly trained endurance athletes, eight of whom were men that performed vigorous endurance training four to five days a week. Subjects were in good health and free of any diseases.

Subjects visited the laboratory on three separate days. The first visit to lab was a familiarization day that allowed the subjects to become acquainted with all equipment and procedures, that way when they come into lab for a trial there will be no surprises for the subjects. Additionally, subjects performed a VO2 max test on a cycle ergometer. This data was collected in order to compare subjects' orthostatic tolerance to their VO2 max in order to investigate a possible relationship.

The remaining two days involved performing a maximal simulated hemorrhage challenge. During one visit the challenge was performed in normothermic conditions while on the other the challenge was performed during heat stress conditions (i.e. after internal temperature was elevated ~ 1.5 °C. The order of the days was performed in a randomized and counter balanced fashion. The hemorrhage was simulated with a lower body negative pressure machine that was sealed at the iliac crest. Heart rate (ECG), beat-by-beat arterial blood pressure (Penaz method), cerebral perfusion as indexed by the blood velocity in the middle cerebral artery (Trans-cranial Doppler), and arterial carbon dioxide tension as indexed by end-tidal carbon dioxide tension ($PETCO_2$) were recorded. The only difference between protocols on

normothermic and heat stress days is the actual heating process (see below). On the heat stress day, subjects provided a urine sample to ensure proper hydration. Proper hydration was defined as less than 1.02 on a urine specific gravity test. If the subject was determined to be dehydrated based on this test, then the study was cancelled and rescheduled for another day. Following the specific gravity test, subjects ingested a telemetry pill, for continuous measurement of internal temperature. This allowed researchers to heat subjects exactly 1.5 degrees Celsius from their starting temperature. To heat the subjects, they were dressed in a water perfused tube-lined suit, and water at 46.5 degrees Celsius was pumped through the suit. After the desired rise in temperature occurred, experimenters began simulating a hemorrhage.

The simulated hemorrhage challenge involved subjects laying in the supine position in the LBNP box. Convertino (2001) and Cooke (2004) used LBNP to simulate hemorrhage, and LBNP is commonly used in exercise physiology research. The test started out at -20 mmHg for 3 min after which the level of suction was incremented by 10 mmHg every 3 min until the onset of syncopla symptoms occurred. Brothers (2011) and Keller (2009) used this protocol to ensure syncopal symptoms develop. Syncopal symptoms were defined as; pallor, diaphoresis, rapid and progressive drop in mean arterial pressure, sustained systolic blood pressure 80 mmHg, and relative bradycardia accompanied with narrowing of pulse pressure. When these physiological changes happen, subjects may experience discomfort and/or dizziness. When this occurred, trials were immediately terminated, and the experiment ended.

Additionally, blood samples were taken at normothermic baseline (i.e. immediately prior to the onset of heat stress) and heat stress baseline (i.e. immediately prior to the onset of LBNP) to measure ANG II. Blood samples were only successfully obtained from ten subjects. Heat stress and normothermic days were randomized to avoid bias. For statistical analysis the subjects

were broken into three groups of orthostatic tolerance. The seven participants who had the largest difference in orthostatic tolerance between the normothermic and heat stress trials were classified as “heat intolerant” and put in the large difference group, the seven participants with the smallest difference between the two trials were classified as “heat tolerant” and were put in the small difference group, and the six in the middle were eliminated for data analysis purposes. Fitness level differences, difference in cumulative stress index (CSI) between thermal conditions, and physiological variables at LBNP-20 mmHg and presyncope were compared using unpaired Student's t-tests. When a significant interaction was found, a Newman-Keuls post hoc analysis was used for all pairwise comparisons. Since blood samples were not collected for all subjects, a Pearson’s correlation analysis was performed on subjects whose blood was drawn, including the seven middle subjects who were eliminated from other statistical analysis, in order to determine the relationship between tolerance to a simulated hemorrhage and plasma ANG II concentration. Differences were considered statistically significant at the alpha level of .05.

Results:

Orthostatic Tolerance

During the normothermic baseline period before each simulated hemorrhage challenge (normothermic and heat stress), subjects had similar values for heart rate (normothermia CSI trial: 58.2 +/- 9.6 beats/min; heat stress: 58.7 +/- 12.1 beats/min, P = 0.59), and mean arterial pressure (normothermia CSI trial: 84.6 +/- 11.9 mmHg; heat stress: 82.2 +/- 10.2 mmHg, P = 0.13). These data indicate that subjects had similar resting cardiovascular responses before data collection of each trial.

Reponses to the maximal graded simulated hemorrhage challenge are shown in Fig. 1. Despite having a markedly higher CSI during normothermia the Large Difference group (LDG): 1,504 +/- 204 mmHg min; Small Difference Group (SDG): 932 +/- 399 mmHg min; P < 0.01), the LDG achieved a lower CSI during heat stress compared with the SDG (LDG: 136 +/- 57 mmHg min; SDG: 412 +/- 235 mmHg min; P = 0.04). Accordingly, the LDG (more dramatically affected by the heat stress) displayed a larger difference in CSI between normothermic and heat stress trials compared with the SDG (least effected by heat stress) (SDG: 520 +/- 230 mmHg min; LDG: 1,369 +/- 240 mmHg min, P < 0.001).

Thermal and Hemodynamic Responses during Normothermia and Heat Stress Baseline Periods

Internal temperature was similar in the SDG and LDG during normothermia and heat stress baseline periods (Table 1; P = 0.19). Heat stress increased internal temperature (main effect of temperature; P > 0.001), and this effect of heat stress was similar between groups (Table 1; interaction P = 0.99). There was no difference in any hemodynamic variable during normothermic baseline between SDG and LDG (Table 1; P > 0.05 for all variables). Although middle cerebral blood velocity (MCA Vmean) and cerebral vascular conductance index (CVCi)

were reduced during heat stress compared with normothermia (Table 1; main effect of temperature for MCA Vmean: $P < 0.001$; main effect of temperature for CVCi: $P < 0.01$), the magnitude of reduction did not differ between groups (Table 1; $P > 0.05$ for both). PETCO2 was reduced during heat stress compared with normothermia (Table 1; main effect of temperature; $P < 0.001$); however, the reduction was similar between the LDG and SDG (Table 1; $P = 0.99$). Heart rate was elevated during heat stress compared with normothermia (Table 1; $P > 0.001$), and there was a significant interaction [group temperature (Table 1); $P = 0.02$], such that heart rate was higher in the LDG group compared with the SDG group during heat stress (Table 1; $P = 0.05$).

Hemodynamic Responses During LBNP20

The only common LBNP stage completed by all individuals in both groups (LDG and SDG) was LBNP20; therefore, hemodynamic variables were compared at this stage in order to obtain further insight into mechanisms resulting in the variability in CSI difference between the SDG and LDG (Fig. 1).

Absolute values for MCA Vmean and PETCO2 were reduced during LBNP20 in the LDG group compared with the SDG group (Fig. 2, A and B, respectively; $P < 0.05$ for both); whereas heart rate values were higher (Fig. 2C; $P = 0.04$) and MAP was similar during LBNP20 in the LDG group compared with the SDG group (Fig. 2; $P = 0.23$). When MCA Vmean data were analyzed as the reserve for cerebral perfusion at LBNP20 (MCA Vmean at LBNP20 MCA Vmean at presyncope), MCA Vmean reserve values were greater in the SDG (Fig. 3; P = 001).

ANG II and Aerobic Fitness

Plasma ANG II increased from normothermia to heat stress as predicted (Fig. 4, $P < 0.01$), but the relationship between the changes in ANG II from normothermia to heat stress was

not correlated with difference in tolerance to a simulated hemorrhage between normothermia and heat stress conditions (normothermic CSI heat stress CSI) (Fig. 4; r = 0.05, P = 0.90). Likewise, values for cardiorespiratory fitness, indexed by VO2peak during cycle ergometry, in absolute terms (l/min; data not shown) and expressed relative to body mass (ml·min 1·kg 1; Fig. 4), were similar in the LDG compared with the SDG (P > 0.05 for both).

Presyncope

Cardiovascular values were similar between the LDG and SDG at presyncope (Table 2, P > 0.05 for all).

Discussion:

This study aimed to expand our knowledge of the underlying mechanisms of orthostatic tolerance. First, cerebral perfusion was compared with reductions in orthostatic tolerance between normal thermic and heated conditions. The researchers' hypothesized that subjects with the greatest reduction in orthostatic tolerance will experience the largest drop in cerebral blood flow. Additionally, ANG II was measured in order to identify if during passive heating, the elevation in plasma ANG II was negatively correlated with heat-stress induced reductions in orthostatic tolerance. Lastly, orthostatic tolerance changes during the simulated hemorrhage between heat stress and normal thermic conditions will be compared to fitness level, measured by VO2 max.

The hypothesis that subjects in the LDG experienced the greatest reduction in cerebral blood flow was not supported. As seen in previous research, Wilson (2006) and Brothers (2011), cerebral perfusion was reduced during heat stress, but there was not a statistical difference between the drop in cerebral perfusion between the LDG and the SDG (Table 2). These results show that variability in tolerance during a simulated hemorrhage test under heat stress is not correlated with heat stress-induced reduction in cerebral perfusion. While heat stress-induced reductions in baseline cerebral perfusion were similar between groups (Table 1), the reduction in cerebral perfusion was larger in the LDG compared with the SDG during the initial stage of the simulated hemorrhage challenge, which resulted in lower values for MCA Vmean (Fig. 2A). Additional analysis of cerebral perfusion at LBNP20 between the LDG and SDG was assessed by determining the cerebral perfusion "reserve" at this stage (MCA Vmean at LBNP20 – MCA Vmean at presyncope). Using this approach, the SDG group had a greater cerebral perfusion "reserve" compared with the LDG (Fig. 3). Taken together, these findings indicate that, during

the initial stage of LBNP, individuals in the LDG had a reduced range for cerebral perfusion to be further reduced before presyncopal signs developed.

There are different mechanisms that may have affected cerebral perfusion during LBNP20. One possibility for the immediate drop in cerebral perfusion during LBNP that was only seen in the LDG could be related to the carbon dioxide (CO2) response during the first stage of the simulated hemorrhage challenge. Cerebral circulation is extremely sensitive to changes in PaCO2, with hypocapnia decreasing cerebral perfusion and hypercapnia increasing cerebral perfusion (Jordan, 2000). In addition to CO2, another mechanism that reduces cerebral perfusion is the activation of the sympathetic nervous system. Heat stress significantly increases sympathetic activity relative to normothermia (Keller, 2006). Also, reduced central blood volume increases sympathetic activity in normothermic conditions and augments the increase during heat stress conditions (Brothers, 2011). Because of this, it is possible that subjects in the LDG may have had more sympathetic activity during the first stage of the simulated hemorrhage challenge. All these mechanisms contribute to the body's cerebral blood flow, which makes it incredibly hard to directly link cerebral blood flow with one mechanism; such as, orthostatic tolerance.

ANG II was measured in order to identify if during passive heating, the elevation in plasma ANG II was negatively correlated with heat-stress induced reductions in orthostatic tolerance. ANG II is one of the most potent vasoactive hormones in humans. It is also important to note that plasma levels of ANG II rise when central blood volume is reduced during normal thermic conditions, and during whole body heat stress (Greenleaf, 2000). The attenuated activation of the RAAS and reduced plasma renin activity occurs when there is reduced blood pressure control and orthostatic tolerance during normal thermic conditions (Fig. 4A) (Jacob,

1997). As seen in figure 4A, the increase in plasma ANG II was not predictive of differences in tolerance during a simulated hemorrhage test between normothermic and heat stress conditions.

Third, subjects' VO2 max was compared with their tolerance on the two simulated hemorrhage tests. Again, VO2 max was found to not be predictive of the differences in tolerance between the two conditions (Fig. 4B). These findings agree with Levine (1991), Mtinangi (1999), and Winker (2005), all of whom found no linear relationship between fitness level and orthostatic tolerance despite the many fitness components related to orthostatic tolerance.

The aim of this study was to investigate the relationship between cerebral perfusion, ANG II, and VO2 max with subjects' difference in tolerance during a simulated hemorrhage test under normothermic conditions and heat stress conditions. Cerebral perfusion was compared with reductions in orthostatic tolerance between normal thermic and heated conditions, and no relationship was found. This means that the reduction in orthostatic tolerance between the two conditions is not related to the drop in cerebral blood flow. Also, both ANG II and VO2 max were not correlated with heat-stress induced reductions in orthostatic tolerance. This study aimed to expand the knowledge of the underlying mechanisms of orthostatic tolerance. It has become abundantly clear that many mechanisms contribute to the differences in cerebral perfusion, and more future research is required to tease out all the underlying mechanisms of orthostatic tolerance. Since the initial stage is the only stage completed by all subjects under both conditions, a specific possibility for future research is to look at the differences between LDG and SDG cerebral perfusion early on during the simulated hemorrhage test instead of at onset of syncope. By doing this, researchers will be able to better look at possible mechanisms that present themselves early on during the test, and then they may compare them under the same LBNP conditions.

Figures

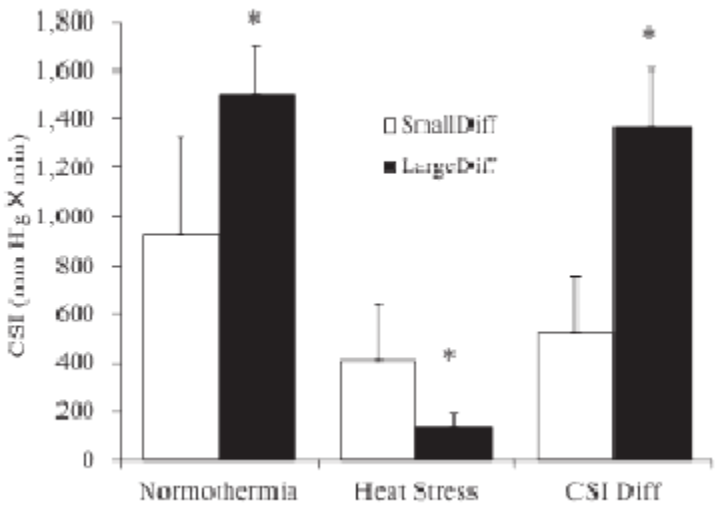

Figure 1: Cumulative stress index (CSI) values during normothermia (NT) and heat stress (HS), and the difference between NT and HS (CSI Diff) for individuals with small differences in tolerance to a simulated hemorrhage between NT and HS (SDG, n = 7) and large differences in tolerance to a simulated hemorrhage between NT and HS (LDG, n = 7). Values are means +/- SD. *Significantly different compared with SDG, $P < 0.05$.

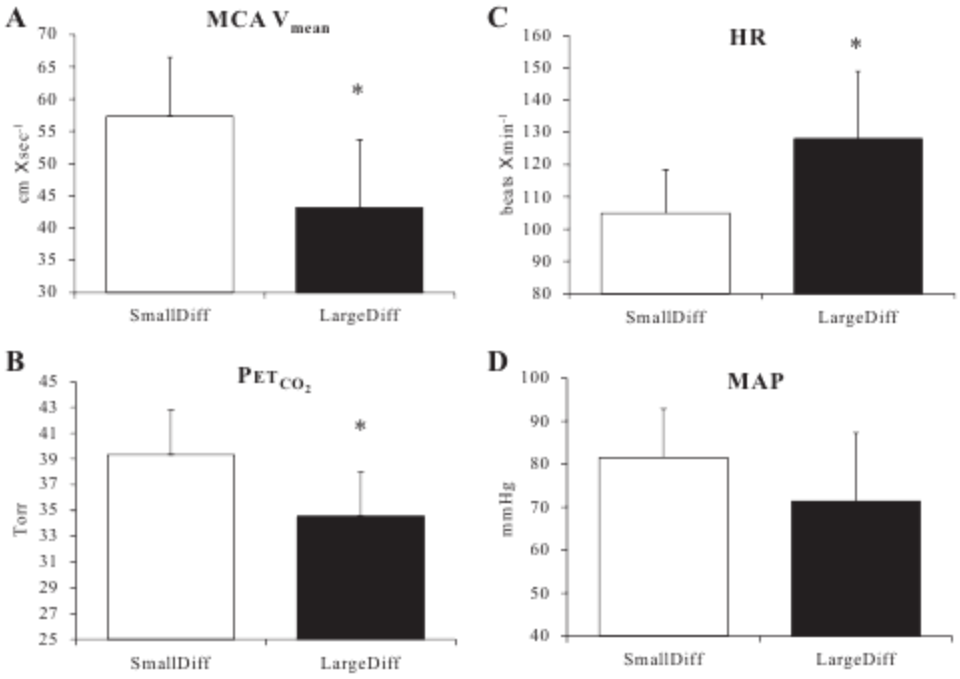

Figure 2: Middle cerebral artery blood velocity (MCA Vmean; A), end-tidal carbon dioxide tension (PETCO2; B), heart rate (HR; C), and mean arterial pressure (MAP; D) during the initial stage of lower body negative pressure (LBNP20). All data shown were collected on the HS day. Values are means +/- SD. *Significantly different compared with SDG, $P < 0.05$.

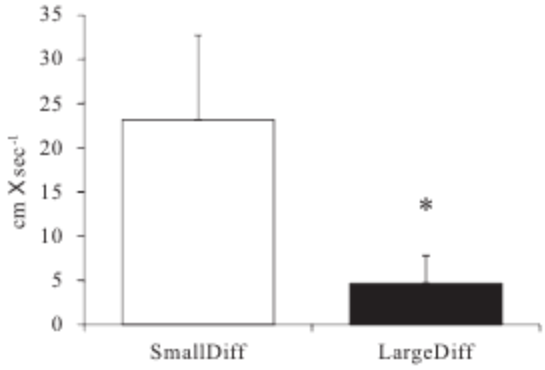

Figure 3: MCA Vmean expressed as the difference between values at LBNP20 and at presyncope (MCA Vmean reserve) for SDG (n = 7) and LDG (n = 7) individuals. Values are means SD.

*Significantly different compared with SDG, P = 0.001.

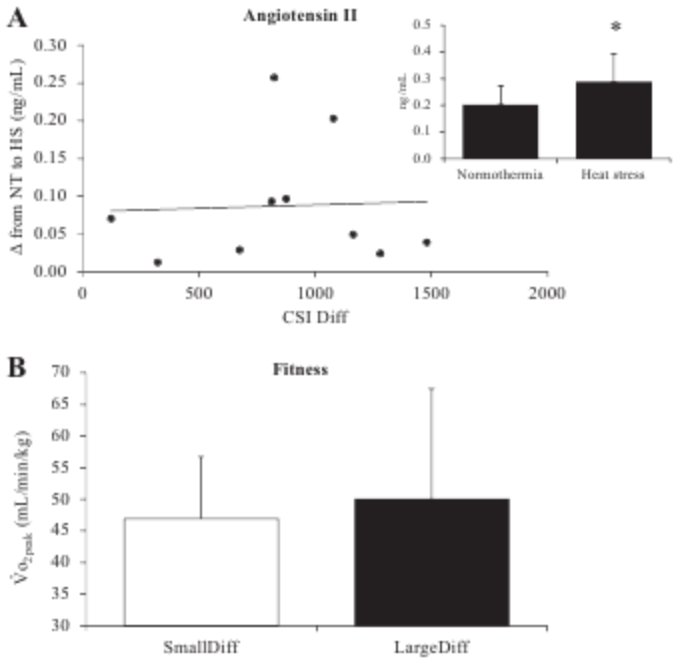

Figure 4: A: relationship between the change in plasma angiotensin II, and the CSI difference. Inset: plasma ANG II values during normotheric and heat stress. B: fitness, indexed as maximal oxygen consumption, SDG (n = 7) and LDG (n =7) individuals. Values are means +/- SD. *Significantly different compared with NT, $P < 0.01$.

Tables

	Normothermia		Heat Stress		
	SmallDiff	LargeDiff	SmallDiff	LargeDiff	*P* Value for Interaction
MCA V_{mean}, cm/s	67.7 ± 5.9	67.2 ± 16.2	53.6 ± 9.7†	48.6 ± 11.6†	0.51
CVCi, cm·s^{-1}·mmHg^{-1}	85.7 ± 17.1	84.3 ± 30.7	70.3 ± 18.4*	58.9 ± 17.2*	0.42
Internal temperature, °C	36.7 ± 0.2	36.9 ± 0.4	38.1 ± 0.3†	38.4 ± 0.3†	0.99
$P_{ET_{CO_2}}$, Torr	45.8 ± 4.0	46.1 ± 2.9	38.9 ± 3.6†	39.2 ± 2.7†	0.99
MAP, mmHg	80.9 ± 10.7	83.8 ± 12.4	79.2 ± 13.4	84.7 ± 11.6	0.66
HR, beats/min	59.6 ± 8.4	65.2 ± 65.2	99.5 ± 13.5†	121.2 ± 22.2†	0.03

Values are means ± SD. MCA V_{mean}, middle cerebral artery blood velocity; CVCi, cerebral vascular conductance index; $P_{ET_{CO_2}}$, end-tidal P_{CO_2}; MAP, mean arterial pressure; HR, heart rate. Significant main effect of thermal condition: †$P < 0.001$ and *$P < 0.05$. Bold value represents a significant (group × temperature interaction).

Table 1: Baseline hemodynamic and thermal data during normothermia and heat stress in SDG and LDG individuals.

	SmallDiff	LargeDiff	*P* Value
MCA V_{mean}, cm/s	33.5 ± 4.3	38.1 ± 9.1	0.32
CVCi, cm·s^{-1}·mmHg^{-1}	50.3 ± 11.8	63.3 ± 19.4	0.22
$P_{ET_{CO_2}}$, Torr	29.5 ± 3.7	29.8 ± 5.6	0.93
MAP, mmHg	68.5 ± 10.0	62.1 ± 8.6	0.26
HR, beats/min	121.0 ± 20.2	121.8 ± 17.9	0.94

Values are means ± SD.

Table 2: Hemodynamic data at presyncopy during heat stress.

References

Brothers, R.M., Keller, D.M., Wingo, J.E., Ganio, M.S., & Crandall, C.G. (2011). Heat-stress-induced changes in central venous pressure do not explain interindividual differences in orthostatic tolerance during heat stress. *Journal of Applied Physiology*, 110, 1283–1289.

Brothers, R.M., Wingo, J.E., Hubing, K.A., & Crandall, C.G. (2009). The effects of reduced end-tidal carbon dioxide tension on cerebral blood flow during heat stress. *Journal of Physiology*, 587, 3921–3927.

Convertino, V.A. (2001). Lower body negative pressure as a tool for research in aerospace physiology and military medicine. *Journal of Gravity and Physiology*, 8, 1–14.

Cooke, W.H., Ryan, K.L., & Convertino, V.A. (2004). Lower body negative pressure as a model to study progression to acute hemorrhagic shock in humans. *Journal of Applied Physiology*, 96, 1249 –1261.

Escourrou, P., Freund, P.R., Rowell, L.B., & Johnson, D.G. (1982). Splanchnic vasoconstriction in heat-stressed men: role of renin-angiotensin system. *Journal of Applied Physiology*, 52, 1438 –1443.

Greenleaf, J.E., Petersen, T.W., Gabrielsen, A., Pump, B., Bie, P., Christensen, N.J., Warberg, J., Videbaek, R., Simonson, S.R., & Norsk, P. (2000). Low LBNP tolerance in men is associated with attenuated activation of the renin-angiotensin system. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, 279, 822–829.

Harrison, M.H., Kravik, S.E., Geelen, G., Keil, L., & Greenleaf, J.E. (1985). Blood pressure and plasma renin activity as predictors of orthostatic intolerance. *Aviation Space Environment Medicine*, 56, 1059 –1064.

Jacob, G., Robertson, D., Mosqueda-Garcia, R., Ertl, A.C., Robertson, R.M., & Biaggioni, I. (1997). Hypovolemia in syncope and orthostatic intolerance role of the renin-angiotensin system. *American Journal of Medicine*, 103, 128 –133.

Jordan, J., Shannon, J.R., Diedrich, A., Black, B., Costa, F., Robertson, D., & Biaggioni, I. (2000). Interaction of carbon dioxide and sympathetic nervous system activity in the regulation of cerebral perfusion in humans. *Hypertension*, 36, 383–388.

Keller, D.M., Cui, J., Davis, S.L., Low, D.A., & Crandall, C.G. (2006). Heat stress enhances arterial baroreflex control of muscle sympathetic nerve activity via increased sensitivity of burst gating, not burst area, in humans. *Journal of Physiology*, 573, 445–451.

Keller, D.M., Low, D.A., Wingo, J.E., Brothers, R.M., Hastings, J., Davis, S.L., & Crandall, C.G. (2009). Acute volume expansion preserves orthostatic tolerance during whole-body heat stress in humans. *Journal of Physiology*, 587, 1131–1139.

Levine, B.D., Buckey, J.C., Fritsch, J.M., Yancy, C.W. Jr., Watenpaugh, D.E., Snell, P.G., Lane, L.D., Eckberg, D.L., & Blomqvist, C.G. (1991). Physical fitness and cardiovascular regulation: mechanisms of orthostatic intolerance. *Journal of Applied Physiology*, 70, 112–122.

Levine, B.D., Lane, L.D., Buckey, J.C., Friedman, D.B., & Blomqvist, C.G. (1991). Left ventricular pressure-volume and frank-starling relations in endurance athletes. Implications for orthostatic tolerance and exercise performance. *Circulation*, 84, 1016 – 1023.

Mtinangi, B., and Hainsworth, R. (1999). Effects of moderate exercise training on plasma volume, baroreceptor sensitivity and orthostatic tolerance in healthy subjects. *Experimental Physiology*, 84, 121–130.

Wilson, T.E., Cui, J., Zhang, R., & Crandall, C.G. (2006). Heat stress reduces cerebral blood velocity and markedly impairs orthostatic tolerance in humans. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, 291, 1443–1448.

Winker, R., Barth, A., Bidmon, D., Ponocny, I., Weber, M., Mayr, O., Robertson, D., Diedrich, A., Maier, R., Pilger, A., Haber, P., & Rudiger, H.W. (2005). Endurance exercise training in orthostatic intolerance: a randomized, controlled trial. *Hypertension*, 45, 391–398.